Abstract

Charles University
Faculty of Pharmacy in Hradec Králové
Department of Pharmacology and Toxicology
Candidate: Daniel Herbolt, MSc.
Supervisor: Assoc. Prof. Přemysl Mladěnka, Pharm.D., Ph.D.
Title of rigorous thesis: Development of a novel spectrophotometric method for zinc ions chelatation screening

Zinc is the only one trace metal, which is a cofactor for over 300 active enzymes. It is localized in all tissues and organs. Zinc deficiency can cause many diseases such as e.g. diabetes, disorders of hemostasis, and cancer. The toxicity of zinc is usually considered to be mild, but the imbalance of zinc, e.g. its presence in extracellular plaques by patients with Alzheimer's disease suggests a more complex relationship. The purpose of this work was to create a new spectrophotometric methodology for screening of chelation of zinc ions.

The first step was to measure the spectra of the selected indicator dithizone and its complex with zinc and finding the ideal wavelengths for accurate determination of the linear concentration of zinc ions. Thereafter, the linearity was checked at two selected wavelengths. After that, the stability of dithizone and its complex with zinc was measured in relationship to time. The final phase was to validate the methodology on two known zinc chelators which were ethylenediaminetetraacetic acid (EDTA) and N,N,N′,N′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN).

Initial measurements showed a shift of the dithizone 's spectrum after adding zinc at all tested values of pH (4.5, 5.5, 6.8 and 7.5). As the most suitable wavelengths for determination of the concentration of zinc were chosen: at pH 4.5 - 530 nm and 570 nm and for pH 5.5, 6.8 and 7.5 - 540 nm and 590 nm. At these wavelengths, a high linearity between the absorbance and the concentration of zinc has been demonstrated. Stability studies have shown the need of using the indicator within 24 hours after preparation. Verification of the method using chelators TPEN and EDTA confirmed its suitability at lower tested wavelengths. Surprisingly, in the case of TPEN,

the measurement at higher wavelengths appeared to be associated with a higher experimental error.

In conclusion, we can say that we managed to standardize the method for the determination of zinc cations, which could be used in future for screening of interaction of various substances with zinc ions *in vitro*.